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EDITORIAL COMMENT

# Contrast-Enhanced Ultrasound and the Enigma of Plaque Neovascularization*

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*"It was thanks to Ultra that we won the war"*
*—Winston Churchill (1)*

The concept that plaque neovascularization may play a role in atherosclerosis is more than a century old. More recently, it has been hypothesized that lesion microvessels augment delivery of immunomodulatory and repair cells and soluble factors (including growth factors and cholesterol) into developing atherosclerotic plaque (reviewed in [2]). These findings have been underscored by clinical data showing an association between plaque angiogenesis and more high-grade lesions. Particular clinical attention has focused on specific aspects of plaque angiogenesis, namely the role of inflammatory cells in augmenting lesion microvasculature, the potential positive feedback loop between angiogenesis and inflammatory cell infiltration into plaque, and most intriguingly, the potential for highly angiogenic lesions to undergo intraplaque hemorrhage. Elucidation of these complex interactions between the plaque structure, cellular composition, and microvascular architecture may ultimately provide key insights into the broader clinical question of plaque stability assessment and predicting future cardiovascular events.

See pages 1256 and 1265

In this issue of *iJACC*, 2 studies highlight ultrasound imaging of atherosclerotic plaque microvasculature (3,4). Atherosclerotic plaque angiogenesis, a feature of advanced pathology, has been reported to be associated with plaque vulnerability (5), both histologically and clinically. Plaque microvessels, prone to leakage, can act as conduits for erythrocytes, inflammatory cells, cytokines, and proteases, in addition to being prone to hemorrhage, all of which potentially contribute towards lesion progression and/or rupture (2). Whether plaque angiogenesis has a causal relationship with lesion instability, or occurs inter alia as a consequence of localized hypoxia within the lesion core has not yet been clarified. Nevertheless, better identification of vascularized plaques through diagnostic imaging techniques is highly desirable from a clinical perspective.

Standard angiographic imaging is currently used to identify and quantify the severity of vascular stenosis, but provides little information on lesion composition. Early studies using serial prospective as well as retrospective coronary angiographic observations suggested that not all acute vascular occlusions and infarctions resulted from severely obstructive plaques (6). Thus, angiography alone is inadequate for prediction of high-risk plaques, and identification of lesions with a high microvessel density may offer additional insight into plaque vulnerability.

Numerous imaging modalities have been used to visualize neovascularization in a clinical setting, including magnetic resonance imaging, positron emission tomography, single-photon emission computed tomography, computed tomography (CT), and planar and intravascular ultrasound (7,8). CT has been utilized for the study of plaque microvessels in experimental atherosclerosis models, with some correlation between contrast signal and microvessel density (9). In the absence of contrast-enhancing agents such as perfused microbubbles, standard planar ultrasound techniques have exhibited poor accuracy in detecting lesion topography and compositional features desired for ascribing a tendency to rupture. A number of recent efforts using contrast enhancement in humans have been made wherein an enhanced contrast signal in the periadventitial vasa vasorum was detected in

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patients with carotid atherosclerosis and correlated with intima-media thickening (10).

In both studies featured in this issue of *iJACC*, plaque vascularity in the rabbit model of high cholesterol–induced atherogenesis was detected using contrast-enhanced ultrasound (CEU) imaging, and in each, improvements in signal processing capability were applied to enhance identification of plaques containing microvessels. In the first study, Giannarelli et al. (3) introduced a scoring system of lesions based on CEU that was related to histological characteristics. Lesions with high-contrast enhancement by ultrasound, upon histological examination, had increased vessel density, macrophage content, and matrix deposition, whereas more fibrotic lesions did not, indicating a correlation between the ultrasound parameters, lesion development, and composition. This contrast enhancement approach also allowed the introduction of a potentially useful scoring system for lesion microvascularity (3).

In the second study, Lee et al. (4) created an intravascular hemorrhage model by injecting autologous blood into the medial/adventitial space that resulted in an expansion of neovessels in the vasa vasorum. The expanded vasa vasorum in blood-injected lesions (that was absent with saline treatment) could be clearly imaged employing CEU. Positive signal for plaque microvasculature was enhanced through use of a maximum intensity projection, in which a maximal pixel intensity following a destructive pulse sequence was recorded, allowing a "trail of enhancement" from contrast agents through the vasa vasorum to be observed. In previous studies comparing CEU with post-mortem histology, the degree of enhancement attained was low compared with the local vascular density (11). Therefore, tracking of microvessels throughout the lesion by maximum intensity projection results in a trace of contrast agents, which in turn renders it more visible. By enhancing the affinity of microbubbles for ligands presented by the locally inflamed microvascular environment, in this case ICAM-1, the tracing of microvessels was even further enhanced (4).

Although both studies represent progress in image processing and histological correlation of a defined ultrasonic signature, a series of challenges remain to be addressed before clinical translation becomes a reality. These methodologies, combining CEU and improved signal processing, will need to be used in serial prospective studies in humans to truly define a comprehensive ultrasound profile of plaques that are perhaps prone to rupture. These promising improvements in signal enhancement in conjunction with CEU can potentially be applied not only to planar, but also to intravascular ultrasound (IVUS) enhancing the current capabilities of vascular imaging (12). Thus, a multifaceted, ultrasound imaging platform capable of providing optimal topograph-

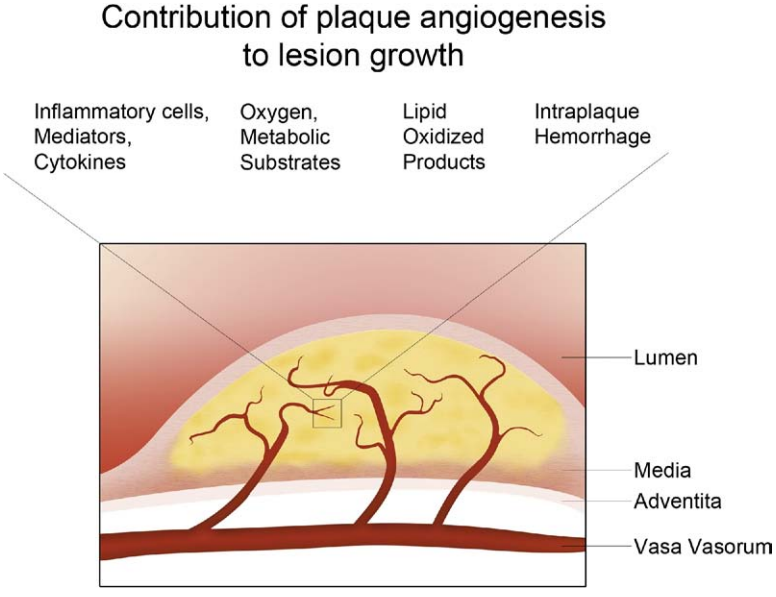


**Figure 1. Vessel Wall Angiogenesis and Atherosclerosis**

Putative role for microvessels in atherosclerotic plaque biology includes augmentation of cellular and soluble lesion components, enhancement of oxygen exchange, delivery of metabolic substrates and lipids, and potentiation of microvascular hemorrhage within the plaque intima.

ical resolution and compositional information on lesions studied may be feasible. The incorporation of CEU with IVUS–virtual histology has been attempted in complex porcine coronary atherosclerotic lesions but IVUS–virtual histology, in this report, did not accurately detect the relative amounts of specific plaque components within each individual corresponding histological specimen (13). It is possible that this failure was a reflection of the inherent limitations of the porcine plaque model. Indeed, it could be speculated that improvements in signal processing in this setting may provide an integrated solution for plaque analysis incorporating detection elements for thrombus-associated plaque, vascularity, lesion dimension, and potentially, vulnerability.

As implied by Giannarelli et al. (3), a lesion classification score based on contrast enhancement although useful, needs to be validated against evidence of plaque rupture as opposed to presumed features of vulnerable plaque, namely microvessel density, macrophage content, or matrix characteristics. The information on plaque composition and dimensions gained from future iterations of CEU needs to also offer advantages over competing imaging techniques. The inclusion of targeted contrast agents used by Lee et al. (4) in which ICAM-1–targeted microbubbles enhanced the detection of vasa vasorum neovessels, has an additional benefit in potentially giving the investigator numerous options for assessing plaque composition.

These studies taken together represent a significant step towards attaining the necessary resolution and compositional information that will make vascularized plaque identification possible. Significant efforts have already been made to develop methods for noninvasive imaging of vascular lesions, incorporating their molecular characteristics, which may improve early diagnostic capabilities and enhance therapeutic decision making (14). The advantage of integrating CEU into existing clinical ultrasound infrastructure may be its use as a low-cost alternative to more expensive imaging modalities such as magnetic resonance imaging or CT. In conclusion, improvements in ultrasound image acquisition, signal processing, and contrast agent enhancement and targeting provide hope that a comprehensive characterization of plaque composition and vulnerability may be achievable. (Fig 1)

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